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MINIREVIEW

Microbial Diversity and Its Relationship to Planetary Protection

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BACKGROUND

As summarized by Rummel (56) and Rummel and Meyer (57), while exploring our solar system and the universe at large, spacefaring nations must be committed to avoiding biological contamination of other planetary systems while also protecting the Earth from potential harm caused by materials returned from space. Most scientists accept this, and there are international treaties and regulations addressing these issues (6, 62). Thus, planetary protection is now a part of planning for all extraterrestrial missions (64), and the rules regarding these activities are prepared by an international group known as the Committee on Space Research (COSPAR) (Paris, France). Spacefaring nations generally adhere to the scientific and technical standards developed by COSPAR.

COSPAR describes five categories for interplanetary missions, and there are suggested ranges of planetary protection requirements for each category. The following descriptions are set forth in the COSPAR regulations (6). Category I includes any mission to a target body that is not of direct interest for understanding the process of chemical evolution or the origin of life; no protection of such bodies is warranted, and no planetary protection requirements are imposed by COSPAR policy. Category II missions are missions whose target bodies are of significant interest relative to the process of chemical evolution and the origin of life but in which there is only a remote chance that contamination carried by a spacecraft could jeopardize future exploration. COSPAR requires only simple documentation that includes preparation of a short planetary protection plan in the form of an outline of intended or potential impact targets, brief pre- and postlaunch analyses detailing impact strategies, and a postencounter and end-of-mission report providing the location of impact, if such an event occurs. Category III missions (mostly flyby and orbiter missions) are missions to a target body of chemical evolution and/or origin-of-life interest or for which scientific opinion indicates that there is a significant chance of contamination that could jeopardize a future biological experiment. COSPAR requires documentation of planetary protection issues and some implementation of protection procedures that include at a minimum trajectory biasing, the use of cleanrooms during spacecraft assembly and testing, and possibly spacecraft bioburden reduction. An inventory of bulk constituent organics is required if the probability of impact is significant. Category IV missions (mostly probe and lander missions) target a body of chemical evolution and/or origin-of life-interest or for which scientific opinion indicates that there is a significant chance of contamination that could jeopardize future biological experiments. COSPAR requires detailed documentation of planetary protection issues, including a bioassay to enumerate spacecraft bioburden, an analysis of the probability of contamination that may include trajectory biasing, use of cleanrooms during spacecraft assembly, bioload reduction, partial sterilization of any direct contact hardware, and a bioshield for that hardware. The requirements and compliance are similar to those imposed for the *Viking* missions, with the exception of complete lander or probe sterilization. Category V comprises all return-to-Earth missions, where the concern is the protection of the terrestrial system comprising the Earth and the Moon. The Moon must be protected from back-contamination to retain freedom from planetary protection requirements for Earth-Moon travel. For solar system bodies deemed by scientific opinion to have no indigenous life forms, an "unrestricted Earth return" subcategory is defined; missions in this subcategory have planetary protection requirements on the outbound phase only that correspond to the category of that phase (typically category I or II). For all other category V missions, in a subcategory defined as "restricted Earth return," the highest degree of concern is expressed by the absolute prohibition of destructive impact upon return, the need for containment throughout the return phase of all returned hardware which directly contacted the target body or unsterilized material from the body, and the need for containment of any unsterilized sample collected and returned to Earth. Postmission, timely analysis of the unsterilized, returned sample(s) is required under strict containment, using the most sensitive techniques. If any sign of a nonterrestrial replicating entity is found, the returned sample must remain contained unless it is treated by an effective sterilizing procedure.

None of the suggested disinfection procedures for categories I to IV actually requires sterilization of the entire spacecraft; however, the COSPAR regulations are often more specific for certain locations and types of missions. For example, if a "special region" of Mars is to be accessed though horizontal or vertical mobility, either the entire landed system must be sterilized to the *Viking* poststerilization biological burden levels or the subsystems which directly contact the special region must be sterilized to these levels, and a method of preventing their recontamination prior to accessing the special region must be provided. If an unplanned condition (e.g., a hard landing) could result in a high probability of inadvertent biological

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contamination of the special region by the spacecraft, the entire landed system must be sterilized to *Viking* poststerilization biological burden levels. COSPAR defines a special region as an area within which terrestrial organisms are likely to propagate or a region which is thought to have a high potential for the existence of extant life forms. Readers who are interested in such details are referred to the COSPAR document (6) and other relevant NASA and National Research Council documents (3–5).

Since scientific investigations using Earth-launched spacecraft frequently target Mars, there are particular and immediate needs for planetary protection planning related to study of this planet. The concern that forward contamination of Mars might complicate the search for extraterrestrial life has been increased by data indicating that there is frequent meteorite exchange between Mars and Earth (25) and by the strong probability that living bacterial spores can survive interplanetary transfer (42, 46). This raises the distinct possibility that Martian life could resemble life on Earth (37). Irrespective of scientific issues, it is also necessary to address public concerns about potential back-contamination of the Earth (5, 19) and ecological contamination of Mars with Earth organisms, as well as to fulfill formal legal requirements of various laws, such as the National Environmental Policy Act that are already in place (50). Unfortunately, there is no universal appreciation of the difficulty of accomplishing these goals given our limited knowledge of microbial ecology and diversity even here on Earth. The fact is that we do not even know the identities of most of the microorganisms that are actually on the surfaces or within the interiors of our spacecraft.

Spacecraft assembly facilities such as those used by NASA at the California Institute of Technology's Jet Propulsion Laboratory (JPL), the Johnson Space Center, and the Kennedy Space Center are unique microbiological environments. They are extremely oligotrophic (nutrient poor and high stress) because they are rigorously and repeatedly cleaned with antimicrobial agents, particulates are continuously filtered from the air circulating through the facilities, the atmospheres within the facilities are maintained at low humidity, and most surfaces are comprised of man-made materials, such as polished metals. Thus, these facilities are highly selective for indigenous communities of microorganisms that resist desiccation, chemical sterilization agents, and high-energy radiation (36, 68). Interestingly, radiation resistance is observed in these communities even though the facilities themselves are not exposed to unusual radiation other than normal lighting. The local sources of microbes, however, are sometimes subject to high solar light intensities, and these sources provide the microbial forms that eventually are subject to the selective pressure of the cleanroom environment (49).

Since we will be searching Mars for extraterrestrial life, this planet is of special concern regarding issues of planetary protection. Therefore, we need to protect Mars from contamination and thereby protect the integrity of future science missions to the planet. As discussed by Barengoltz (8) and others (30, 39), future sample acquisition flight missions to Mars pose a number of specific protection issues. There is concern for contamination of Earth by possible Mars organisms (19). Also, there is a need for robust anticontamination procedures for the forward protection of Mars and for the sake of future missions. We clearly need to ensure that terrestrial microbes from acquisition missions do not contaminate samples analyzed in situ or after return to Earth. The microbial forms that survive within spacecraft assembly facilities can potentially contaminate spacecraft assembled in them and thus ultimately their destinations.

It has been known since the *Viking* missions, in which dry-heat sterilization of the spacecraft was employed (48, 49), and from research done since then in various world laboratories (66) that Earth microorganisms may significantly contaminate space-qualified materials. It also is recognized that endospores are of special concern. The moderate levels of dry heat or chemical disinfectants that do not harm the spacecraft or its instruments often are insufficient to kill endospores (67). To complicate matters, recent work at JPL characterizing numerous cultivable bacteria from spacecraft assembly facility environments showed that these bacteria have unusual resistance to both physical and chemical antimicrobial agents (36, 68–70). For example, Link et al. (37) identified spores of *Bacillus pumilus* as major culturable bacterial contaminants found on and around spacecraft within the spacecraft assembly facility at the NASA Jet Propulsion Laboratory. One strain, *B. pumilus* SAFR-032, exhibited the highest degree of spore UV resistance observed for any *Bacillus* spp. encountered to date. The observed cultivable strains were mostly gram-positive strains dominated by *Bacillus* species; however, preliminary work using molecular tools (e.g., characterization of numerous 16S rRNA genes amplified by PCR from JPL assembly facility community DNA; analyses of ATP, lipopolysaccharide, and ribosomal or spore-specific DNA) indicated that many uncultivable and as-yet-unstudied bacteria also are present in spacecraft assembly environments (35, 68). These microorganisms, as demonstrated by their presence in these harsh environments, must have characteristics similar to those of cultivated, environmentally robust microorganisms. The uncultivated forms include numerous uncultivated gram-negative strains. Thus, the true diversity of these uncultivable communities has not been assessed. This is a distinct limitation in our present knowledge base that must be surmounted in order to achieve a scientifically valid program for planetary protection.

The particular concern about the potential for survival of *Bacillus* spores on Mars was examined by Schuerger et al. (58). These authors conducted experiments in a Mars simulation chamber to characterize the survival of endospores of *Bacillus subtilis* deposited on aluminum coupons and exposed to high UV irradiation and simulated Martian conditions. The variables examined were pressure, gas composition, and temperature alone or in combination with Mars-normal UV–visible–near-infrared light environments. The authors' data indicated that more than 99.9% of the bacterial populations on sun-exposed surfaces of a spacecraft are likely to be inactivated within a few minutes on the surface of Mars and that within one Mars day, the bacterial populations on sun-exposed surfaces of a spacecraft would be sterilized, thereby minimizing the prospect of forward contamination of Mars by contaminated spacecraft. These types of data are very encouraging regarding the potential for minimizing possible forward contamination of extreme extraterrestrial locations, such as Mars; however, some spores are much more resistant to Mars-like conditions that those of *B. subtilis*, and many areas of a lander

may not be fully exposed to sunlight while on Mars. Thus, more research similar to that of Schuerger et al. (58) but employing more resistant spores (37) is needed.

TOTAL MICROBIAL COMMUNITY ANALYSES

Until recently, the ability to monitor microbial community structure was limited by the lack of suitable means to define species composition and the relative abundance of specific populations in microbial communities. Efforts to do these things have historically relied on culture-dependent methods (11, 24, 32, 44, 72) that are intrinsically limited because the vast majority of bacterial populations in natural communities are refractory to cultivation (33). The accepted protocols for determining microbial burdens on spacecraft surfaces have not been changed in 25 years. The NASA standard assay (31) is used for enumeration of spores and heterotrophic microbial populations. This assay is based on viable counting techniques, such as washing of surfaces with a sterile phosphate-buffered rinse solution with mild sonication, after which the rinse solution is aseptically analyzed for numbers of microbes by standard pour plate techniques using media such as tryptic soy agar. These protocols clearly cannot access the large number of microorganisms in most environments, including spacecraft surfaces, that are presently uncultivable using standard media. For this reason attempts to characterize the structure of even a single community can at best provide only a biased and incomplete view. Moreover, these methods are extremely laborious, and so for practical reasons it has been virtually impossible to do extensive studies to assess changes in communities over time, between locations, or in response to changes in environmental conditions.

There has been some progress in devising means to isolate some previously uncultivated bacteria. For example, Kaeberlein et al. (33) designed diffusion chambers that allowed the growth of previously uncultivated microorganisms in a simulated natural environment. These isolates did not grow on artificial media alone but formed colonies in the presence of other microorganisms. Although this method is less likely to produce results for dry and sparse spacecraft assembly facility communities, as opposed to the marine communities used by Kaeberlein et al. (33), it is a straightforward approach that could be employed in other systems. Stevenson et al. (63) used what they termed an integrative approach to obtain pure cultures of previously uncultivated *Acidobacteria* and *Verrucomicrobia* from agricultural soil and from the guts of wood-feeding termites. The techniques used included the use of agar media with little or no added nutrients, long periods of incubation (>30 days), protection of cells from peroxides, and inclusion of humic acids or a humic acid analogue and quorum-signaling compounds in growth media. However, even the approaches of Kaeberlein et al. (33) and Stevenson et al. (63) ultimately can allow observation of only a very small fraction of the types of bacterial species that are actually present in nature, which probably number in the millions (20).

Fortunately, these limitations on characterization of microbial communities have been overcome to a significant degree through the development of methods that do not rely on cultivation of microbial populations but instead are based on analysis of 16S and 18S rRNA gene sequences that are found in all living organisms. In recent years these methods have been widely employed to explore microbial diversity in diverse microbial habitats and to characterize organisms that have not been cultured yet. The basic strategy has been to use total DNA isolated from a microbial community (metagenomic DNA) as a template for PCR amplification of 16S and 18S rRNA genes using universal primers or primers that are specific for various phylogenetic domains. This is typically followed by construction of an rRNA gene clone library and analysis of individual rRNA gene clones by sequencing or by assessment of restriction fragment length polymorphisms (14) and subsequently determining the phylogeny of the constituent populations. Liu et al. (38) developed a technique called terminal restriction fragment length polymorphism analysis that extends and simplifies this approach by obviating the need to construct a clone library. Briefly, rRNA genes are obtained from total community DNA as described above, except that one of the primers used is labeled with a fluorescent dye. The mixture of rRNA genes is then digested with restriction enzymes that have 4-bp recognition sites, and the size and relative abundance of each fluorescently labeled terminal restriction fragment are determined using an automated DNA sequencer. Since a single fragment represents each numerically dominant member of the community, nominal estimates of diversity within communities can readily be obtained. The pattern of terminal restriction fragments observed (referred to as the "community fingerprint") is a composite of the number of fragments with unique lengths, and the relative abundance of each fragment is roughly reflected in the size of each peak in the electropherogram. Other techniques for microbial community profiling include denaturing gradient gel electrophoresis, temperature gradient gel electrophoresis, single-strand conformation polymorphism analysis, amplified rRNA gene restriction analysis, and amplified intergenic spacer analysis (2).

An assessment of microbial diversity within spacecraft assembly facilities using these modern tools is critically needed to provide basic knowledge concerning the diversity of microorganisms that might contaminate spacecraft that visit non-Earth planetary systems. Less than 1% of the microorganisms present in most natural environments have been grown in pure culture (33). This is almost certainly also true of spacecraft assembly facilities, as the preliminary data of Venkateswaran et al. (69, 70) indicate. Current international treaties and United States regulations require enumeration of cultivable microorganisms but not identification. Previous NASA studies of cultivable strains have provided only a partial and inadequate picture of the microbial community structure within spacecraft assembly facilities. As has been the case in Antarctica (40), there are concerns of possible anthropogenic contamination of Mars, Titan, Europa, and other locations with Earth-derived organic contaminants or life forms that might (i) colonize or survive in their new location and/or (ii) complicate later searches for extraterrestrial life forms. The former is possible since the microbes of spacecraft assembly facilities are known to be highly adapted to extreme environmental conditions, even those that might be encountered on other planets or their moons (69, 70). The latter is possible since the most environmentally robust life forms that may have evolved in extraterrestrial locations may be, as on Earth, microbial. It will be difficult to exclude Earth-derived contamination as a source

of observed microbes if we do not thoroughly understand the complete composition of communities that spacecraft might inadvertently deliver.

Colleagues and I examined the potential for survival of *B. subtilis* endospores on Mars that could some day be inadvertently delivered to that planet as contaminants aboard a spacecraft (15). Mars, which is thought to have highly oxidizing soil, is considered by many researchers to be completely inhospitable to life as we know it on Earth. If so, this would make delivery of Earth microbes and even spores to Mars of little concern. Thus, we examined the hypothesis that if the soil of Mars contains iron as ferrate VI (26), it is self-sterilizing. Ferrate is one of the strongest oxidants known and exists on Earth only in the laboratory. We incubated dried endospores of *B. subtilis* in a Mars surrogate soil comprised of dry silica sand containing 20% (by weight) synthetic ferrate dianion (FeO_4^{2-}), but we used incubation conditions similar to those present on Mars. These conditions included extreme desiccation, high levels of surface UV radiation, cold, and a CO_2-dominated atmosphere. The endospores were not killed and were very resistant to inactivation by the oxidant-enriched sand, even in the presence of high fluxes of sterilizing UV radiation, as long as they were protected by a shallow layer of sand (15). Similar results were observed with permanganate, another strong oxidant. Ronto et al. (55) pointed out that the current Martian UV environment is still quite severe from a biological viewpoint but also showed that substantial protection can be afforded to microbial spores under dust and ice. Based on these data and previously published (controversial) descriptions of ancient but dormant life forms on Earth (13, 22, 27, 28, 43, 71), we concluded that if highly resistant endospores such as those studied at JPL (36, 68, 69) were delivered to Mars, they may remain viable for many years or even indefinitely. Considering the spores within the JPL spacecraft assembly facility that we know about, which are far more robust than *B. subtilis* spores, we clearly need to understand the true diversity of all microbial forms present, even the forms we cannot grow.

CONCLUSIONS

Given our present lack of knowledge of the microbial diversity within spacecraft assembly facilities, what should be the scientific community's research objectives to fill in this missing information? The overall objective of a research program, at a minimum, should be to determine and compare the phylogenetic diversities of microbial communities within the extreme oligotrophic environments of several spacecraft assembly facilities. This research should primarily employ modern molecular and genomics-based tools, since these methods represent the present state of the art for characterizing both the cultivable and uncultivable microbes in a community. At least one spacecraft facility should be representative of a facility located in an arid geographic region. Another should be representative of a spacecraft assembly facility located in a subtropical marine coastal environment. These environments are representative of locations where NASA assembles spacecraft immediately prior to launch. It should be expected that the microbial communities of these types of facilities would be different, reflecting the sources of microbes from the local environments (49).

Although present molecular biology-based tools provide powerful techniques for characterizing microbial community structure and diversity, it must be recognized that even the best of these tools have limitations. There are challenges in extraction of truly representative DNA pools from many environments, and the extracted DNA must be free of contaminants that inhibit enzymes (e.g., *Taq* DNA polymerase) that are used in subsequent operations, such as PCR and cloning (65). Thus, DNA extraction protocols must be carefully examined for individual environments to maximize DNA yields. Sample size must be considered, particularly when microbial populations are small and/or dispersed (52). PCR techniques can have biases that must be recognized, and choices of PCR primers must be made carefully to minimize preferential amplification of some templates (2, 7). It is also sometimes difficult to determine if DNA isolated from a specific environment is derived from dead or living cells. Although it is becoming possible to overcome or minimize some of these known limitations of molecular biology-based metagenomic techniques (35), we must recognize that there likely will be unknown members of many microbial communities that will resist detection by the characterization methods developed to date. Undoubtedly, this will also be true for our spacecraft. However, in characterizing the microbial communities that we are sending into space, it will behoove us to use state-of-the-art methods. Improved methods should be adopted quickly as they are proven in the field.

Preliminary data from the study of microorganisms isolated from the JPL spacecraft assembly facility indicate that microbial strains that survive in this environment are unusually resistant to desiccation, H_2O_2, UV light, and gamma radiation (36, 68–70). If we can understand the mechanisms of these types of resistance at the fundamental level of genes and proteins, space scientists will be able to use this information to make appropriate changes in cleaning technologies to defeat microbial survival. Therefore, some emphasis should be placed on fundamental research on the genetics and structural biology of the unique microbes found in spacecraft assembly areas.

Since strains of bacteria that survive in the spacecraft assembly facility environment almost certainly reflect robust members of the surrounding environment, more research on the microbial diversity of areas near assembly facilities is needed. For example, the environment near JPL is dominated by arid land. Cultivable bacteria found in JPL spacecraft assembly facilities are dominated by *Bacillus* species, and not surprisingly, strains of *Bacillus* appear to be common in desert soils (1, 34, 45, 53, 54). The species that have been observed include *B. subtilis*, *Bacillus stearothermophilus*, and a new species, *Bacillus mojavensis*. However, actinomycetes also are frequently isolated from desert environments, and *Streptomyces* species are observed most often (16, 18, 21, 29, 41, 51). Actinomycetes, however, have yet to be well described for spacecraft assembly facilities. They are almost certainly present, as indicated by culture-independent techniques that have revealed the presence of many gram-positive and gram-negative microorganisms, as well as actinomycetes and fungi (35). Cyanobacteria have received considerable attention as inhabitants of desert environments, where they are found in sun-impacted soil crusts and endolithically in rocks (9, 10, 12, 23, 47). These photosynthetic, chemoautotrophic microbial forms might also find their

way into the lighted, oligotrophic environments of spacecraft assembly facilities, where they might be well adapted for survival; these microbial forms await investigation as part of the community of spacecraft assembly facilities and/or on spacecraft surfaces.

Since many microbial forms appear to be resistant to present sterilization technologies, novel sterilization procedures should be examined. This area has been the focus of some research, but modern instrument packages are not sufficiently robust to withstand some of the previous sterilization treatments, such as use of strong chemicals, penetrating radiation, and heat treatment of spacecraft parts and components carried out before the final assembly of the spacecraft. Likewise, the use of plasmas or gaseous radiation sterilization of the whole spacecraft, as sometimes employed presently (17), needs improvement or replacement. The ultimate objective of such a research program would be to allow sterilization (not just to clean to a specified level) of a complete spacecraft without damage to its structure or instrument packages.

In summary, as discussed by scientists such as Rummel (56), Mancinelli (39), and Horneck et al. (30), planetary protection issues of great importance include minimization of the inevitable deposition of Earth microbes by humans on the surface of Mars or other potentially life-bearing locations in our solar system (59) and prevention of Martian subsurface contamination by Earth microbes and organic material. The natural environments of places in our solar system that may harbor life or complex forms of organic chemicals should be protected so that they retain their value for scientific purposes as humans design planetary missions to search for organic material (60) on and beneath the surface of other planets or to study the chemistry and mineralogy (61) of extraterrestrial landing sites.

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REFERENCES

1. **al-Awadhi, S., S. G. Welch, K. E. Smith, and R. A. Williams.** 1998. BstB7SI (R decreases CCGGY), a thermostable isoschizomer of Cfr10I, from a strain of *Bacillus stearothermophilus* isolated from oil-contaminated soil in Kuwait. FEMS Microbiol. Lett. **160:**205–208.
2. **Anderson, I. C., and J. W. Cairney.** 2004. Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. Environ. Microbiol. **6:**769–779.
3. **Anonymous.** 1992. Biological contamination of Mars issues and recommendations. Space Studies Board, National Research Council, National Academy Press, Washington, DC.
4. **Anonymous.** 1995. Policy guide 8020.12B. NASA, Washington, DC.
5. **Anonymous.** 1997. Mars sample return issues and recommendations. Space Studies Board, National Research Council, National Academy Press, Washington, DC.
6. **Anonymous.** 2002. COSPAR planetary protection policy. COSPAR/IAU Workshop on Planetary Protection. Committee on Space Research (COSPAR), International Council for Science, Paris, France.
7. **Baker, G. C., and D. A. Cowan.** 2004. 16 S rDNA primers and the unbiased assessment of thermophile diversity. Biochem. Soc. Trans. **32:**218–221.
8. **Barengoltz, J. B.** 2000. Planetary protection issues for Mars sample acquisition flight projects. Adv. Space Res. **26:**1911–1916.
9. **Billi, D., E. I. Friedmann, R. F. Helm, and M. Potts.** 2001. Gene transfer to the desiccation-tolerant cyanobacterium *Chroococcidiopsis.* J. Bacteriol. **183:** 2298–2305.
10. **Billi, D., E. I. Friedmann, K. G. Hofer, M. G. Caiola, and R. Ocampo-Friedmann.** 2000. Ionizing-radiation resistance in the desiccation-tolerant cyanobacterium *Chroococcidiopsis.* Appl. Environ. Microbiol. **66:**1489–1492.
11. **Bogardt, A. H., and B. B. Hemmingsen.** 1992. Enumeration of phenanthrene-degrading bacteria by an overlayer technique and its use in evaluation of petroleum-contaminated sites. Appl. Environ. Microbiol. **58:**2579–2582.
12. **Campbell, S. E.** 1979. Soil stabilization by a prokaryotic desert crust: implications for Precambrian land biota. Origins Life **9:**335–348.
13. **Cano, R. J., and M. K. Borucki.** 1995. Revival and identification of bacterial spores in 25- to 40-million-year-old Dominican amber. Science **268:**1060–1064.
14. **Costello, A. M., and M. E. Lidstrom.** 1999. Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. Appl. Environ. Microbiol. **65:**5066–5074.
15. **Crawford, R. L., A. Paszczynski, and L. Allenbach.** 2003. Potassium ferrate [Fe(VI)] does not mediate self-sterilization of a surrogate Mars soil. BMC Microbiol. **3:**4.
16. **Davaadorzh, B., L. P. Terekhova, B. Tsetseg, A. V. Laiko, and T. Puntsag.** 1993. *Streptomyces phaeofaciens*, strain 51—producer of a novel antibiotic of the aureolic acid group. Antibiot. Khimioter. **38:**11–14.
17. **Demidov, V. V., A. A. Goncharov, V. B. Osipov, and V. I. Trofimov.** 1995. Modern aspects of planetary protection and requirements to sterilization of space hardware. Adv. Space Res. **15:**251–255.
18. **Diab, A., and Z. A. Al.** 1976. Actinomycetes in the desert of Kuwait. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. **131:**545–554.
19. **DiGregorio, B. E.** 2001. The dilemma of Mars sample return. Chem. Innov. **31:**18–27.
20. **Dykhuizen, D. E.** 1998. Santa Rosalia revisited: why are there so many species of bacteria? Antonie Leeuwenhoek **73:**25–33.
21. **Eppard, M., W. E. Krumbein, C. Koch, E. Rhiel, J. T. Staley, and E. Stackebrandt.** 1996. Morphological, physiological, and molecular characterization of actinomycetes isolated from dry soil, rocks, and monument surfaces. Arch. Microbiol. **166:**12–22.
22. **Fish, S. A., T. J. Shepherd, T. J. McGenity, and W. D. Grant.** 2002. Recovery of 16S ribosomal RNA gene fragments from ancient halite. Nature **417:**432–436.
23. **Friedmann, E. I.** 1980. Endolithic microbial life in hot and cold deserts. Origins Life. **10:**223–235.
24. **Geiselbrecht, A. D., R. P. Herwig, J. W. Deming, and J. T. Staley.** 1996. Enumeration and phylogenetic analysis of polycyclic aromatic hydrocarbon-degrading marine bacteria from Puget Sound sediments. Appl. Environ. Microbiol. **62:**3344–3349.
25. **Gladman, B. J., J. A. Burns, M. Duncan, P. Lee, and H. F. Levinson.** 1996. The exchange of impact ejecta between the terrestrial planets. Science **271:** 1387–1392.
26. **Goldfield, M., K. Tsapin, and K. Nealson.** 2000. Surface-possible mechanisms and implications. First Astrobiology Science Conference, NASA Ames Research Center, CA Poster Section 12: Mars oxidants.
27. **Greenblatt, C. L., J. Baum, B. Y. Klein, S. Nachshon, V. Koltunov, and R. J. Cano.** 2004. *Micrococcus luteus*—survival in amber. Microb. Ecol. **48:**120–127.
28. **Greenblatt, C. L., A. Davis, B. G. Clement, C. L. Kitts, T. Cox, and R. J. Cano.** 1999. Diversity of microorganisms isolated from amber. Microb. Ecol. **38:**58–68.
29. **Hashem, M., and A. Diab.** 1973. Some physiological studies on *Streptomyces nasri* sp. nov. from the desert of Kuwait. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. **128:**356–362.
30. **Horneck, G., R. Facius, G. Reitz, P. Rettberg, C. Baumstark-Khan, and R. Gerzer.** 2003. Critical issues in connection with human missions to Mars: protection of and from the Martian environment. Adv. Space Res. **31:**87–95.
31. **Jet Propulsion Laboratory.** 1980. NASA standard procedures for the microbiological examination of space hardware. JPL Communication NHB 5340.1B. Office of Space Science, National Aeronautics and Space Administration, Washington, D.C.
32. **Johnsen, K., S. Andersen, and C. S. Jacobsen.** 1996. Phenotypic and genotypic characterization of phenanthrene-degrading fluorescent *Pseudomonas* biovars. Appl. Environ. Microbiol. **62:**3818–3825.
33. **Kaeberlein, T., K. Lewis, and S. S. Epstein.** 2002. Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. Science **296:**1127–1129.
34. **Kamal, M., J. O. Hoog, R. Kaiser, J. Shafqat, T. Razzaki, Z. H. Zaidi, and H. Jornvall.** 1995. Isolation, characterization and structure of subtilisin from a thermostable *Bacillus subtilis* isolate. FEBS Lett. **374:**363–366.
35. **La Duc, M. T., R. Kern, and K. Venkateswaran.** 2004. Microbial monitoring of spacecraft and associated environments. Microb. Ecol. **47:**150–158.
36. **La Duc, M. T., M. Satomi, and K. Venkateswaran.** 2004. *Bacillus odysseyi* sp. nov., a round-spore-forming bacillus isolated from the Mars Odyssey spacecraft. Int. J. Syst. Evol. Microbiol. **54:**195–201.
37. **Link, L., J. Sawyer, K. Venkateswaran, and W. Nicholson.** 2004. Extreme spore UV resistance of *Bacillus pumilus* isolates obtained from an ultraclean spacecraft assembly facility. Microb. Ecol. **47:**159–163.
38. **Liu, W. T., T. L. Marsh, H. Cheng, and L. J. Forney.** 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl. Environ. Microbiol. **63:**4516–4522.

39. **Mancinelli, R. L.** 2003. Planetary protection and the search for life beneath the surface of Mars. Adv. Space Res. **31:**103–107.
40. **Mazzera, D., T. Hayes, D. Lowenthal, and B. Zielinska.** 1999. Quantification of polycyclic aromatic hydrocarbons in soil at McMurdo Station, Antarctica. Sci. Total Environ. **229:**65–71.
41. **Mevs, U., E. Stackebrandt, P. Schumann, C. A. Gallikowski, and P. Hirsch.** 2000. *Modestobacter multiseptatus* gen. nov., sp. nov., a budding actinomycete from soils of the Asgard Range (Transantarctic Mountains). Int. J. Syst. Evol. Microbiol. 50 Pt. **1:**337–346.
42. **Mileikowsky, C., F. A. Cucinotta, J. W. Wilson, B. Gladman, G. Horneck, L. Lindegren, J. Melosh, H. Rickman, M. Valtonen, and J. Q. Zheng.** 2000. Natural transfer of viable microbes in space. Icarus **145:**391–427.
43. **Mormile, M. R., M. A. Biesen, M. C. Gutierrez, A. Ventosa, J. B. Pavlovich, T. C. Onstott, and J. K. Fredrickson.** 2003. Isolation of *Halobacterium salinarum* retrieved directly from halite brine inclusions. Environ. Microbiol. **5:**1094–1102.
44. **Mueller, J. G., R. Devereux, D. L. Santavy, S. E. Lantz, S. G. Willis, and P. H. Pritchard.** 1997. Phylogenetic and physiological comparisons of PAH-degrading bacteria from geographically diverse soils. Antonie Leeuwenhoek **71:**329–343.
45. **Nicholson, W. L., and J. F. Law.** 1999. Method for purification of bacterial endospores from soils: UV resistance of natural Sonoran Desert soil populations of *Bacillus* spp. with reference to *B. subtilis* strain 168. J. Microbiol. Methods **35:**13–21.
46. **Nicholson, W. L., N. Munakata, G. Horneck, H. J. Melosh, and P. Setlow.** 2000. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microbiol. Mol. Biol. Rev. **64:**548–572.
47. **Page-Sharp, M., C. A. Behm, and G. D. Smith.** 1999. Involvement of the compatible solutes trehalose and sucrose in the response to salt stress of a cyanobacterial *Scytonema* species isolated from desert soils. Biochim. Biophys. Acta **1472:**519–528.
48. **Puleo, J. R., S. L. Bergstrom, J. T. Peeler, and G. S. Oxborrow.** 1978. Thermal resistance of naturally occurring airborne bacterial spores. Appl. Environ. Microbiol. **36:**473–479.
49. **Puleo, J. R., N. D. Fields, S. L. Bergstrom, G. S. Oxborrow, P. D. Stabekis, and R. Koukol.** 1977. Microbiological profiles of the Viking spacecraft. Appl. Environ. Microbiol. **33:**379–384.
50. **Race, M. S.** 1996. Planetary protection, legal ambiguity and the decision making process for Mars sample return. Adv. Space Res. **18:**345–350.
51. **Radwan, S. S., G. Barabas, N. A. Sorkhoh, S. Damjanovich, I. Szabo, J. Szollosi, J. Matko, A. Penyige, T. Hirano, and I. M. Szabo.** 1998. Hydrocarbon uptake by *Streptomyces.* FEMS Microbiol. Lett. **169:**87–94.
52. **Ranjard, L., D. P. Lejon, C. Mougel, L. Schehrer, D. Merdinoglu, and R. Chaussod.** 2003. Sampling strategy in molecular microbial ecology: influence of soil sample size on DNA fingerprinting analysis of fungal and bacterial communities. Environ. Microbiol. **5:**1111–1120.
53. **Roberts, M. S., L. K. Nakamura, and F. M. Cohan.** 1994. *Bacillus mojavensis* sp. nov., distinguishable from *Bacillus subtilis* by sexual isolation, divergence in DNA sequence, and differences in fatty acid composition. Int. J. Syst. Bacteriol. **44:**256–264.
54. **Roberts, M. S., L. K. Nakamura, and F. M. Cohan.** 1996. *Bacillus vallismortis* sp. nov., a close relative of *Bacillus subtilis*, isolated from soil in Death Valley, California. Int. J. Syst. Bacteriol. **46:**470–475.
55. **Ronto, G., A. Berces, H. Lammer, C. S. Cockell, G. J. Molina-Cuberos, M. R. Patel, and F. Selsis.** 2003. Solar UV irradiation conditions on the surface of Mars. Photochem. Photobiol. **77:**34–40.
56. **Rummel, J. D.** 2000. Implementing planetary protection requirements for sample return missions. Adv. Space Res. **26:**1893–1899.
57. **Rummel, J. D., and M. A. Meyer.** 1996. A consensus approach to planetary protection requirements: recommendations for Mars lander missions. Adv. Space Res. **18:**317–321.
58. **Schuerger, A. C., R. L. Mancinelli, R. G. Kern, L. J. Rothschild, and C. P. McKay.** 2003. Survival of endospores of *Bacillus subtilis* on spacecraft surfaces under simulated Martian environments: implications for the forward contamination of Mars. Icarus **165:**253–276.
59. **Schulze-Makuch, D., and L. N. Irwin.** 2002. Reassessing the possibility of life on Venus: proposal for an astrobiology mission. Astrobiology **2:**197–202.
60. **Sims, M. R., C. T. Pillinger, I. P. Wright, J. Dowson, S. Whitehead, A. Wells, J. E. Spragg, G. Fraser, L. Richter, H. Hamacher, A. Johnstone, N. P. Meredith, C. de la Nougerede, B. Hancock, R. Turner, S. Peskett, A. Brack, J. Hobbs, M. Newns, A. Senior, M. Humphries, H. U. Keller, N. Thomas, J. S. Lingard, and T. C. Ng.** 1999. Beagle 2: a proposed exobiology lander for ESA's 2003 Mars Express mission. Adv. Space Res. **23:**1925–1928.
61. **Squyres, S. W., R. E. Arvidson, J. F. Bell III, J. Bruckner, N. A. Cabrol, W. Calvin, M. H. Carr, P. R. Christensen, B. C. Clark, L. Crumpler, D. J. Des Marais, C. D'Uston, T. Economou, J. Farmer, W. Farrand, W. Folkner, M. Golombek, S. Gorevan, J. A. Grant, R. Greeley, J. Grotzinger, L. Haskin, K. E. Herkenhoff, S. Hviid, J. Johnson, G. Klingelhofer, A. Knoll, G. Landis, M. Lemmon, R. Li, M. B. Madsen, M. C. Malin, S. M. McLennan, H. Y. McSween, D. W. Ming, J. Moersch, R. V. Morris, T. Parker, J. W. Rice, Jr., L. Richter, R. Rieder, M. Sims, M. Smith, P. Smith, L. A. Soderblom, R. Sullivan, H. Wanke, T. Wdowiak, M. Wolff, and A. Yen.** 2004. The Spirit Rover's Athena science investigation at Gusev Crater, Mars. Science **305:**794–799.
62. **Sterns, P. M., and L. I. Tennen.** 1995. Legal aspects of planetary protection for Mars missions. Adv. Space Res. **15:**281–284.
63. **Stevenson, B. S., S. A. Eichorst, J. T. Wertz, T. M. Schmidt, and J. A. Breznak.** 2004. New strategies for cultivation and detection of previously uncultured microbes. Appl. Environ. Microbiol. **70:**4748–4755.
64. **Sweetser, T. H., C. A. Halsell, and R. J. Cesarone.** 1995. Designing planetary protection into the Mars Observer mission. Adv. Space Res. **15:**257–260.
65. **Tebbe, C. C., and W. Vahjen.** 1993. Interference of humic acids and DNA extracted directly from soil in detection and transformation of recombinant DNA from bacteria and a yeast. Appl. Environ. Microbiol. **59:**2657–2665.
66. **Vasin, V. B., and V. I. Trofimov.** 1995. The experimental study of microbial contamination of the space hardware. Adv. Space Res. **15:**273–276.
67. **Venkateswaran, K., S. Chung, J. Allton, and R. Kern.** 2004. Evaluation of various cleaning methods to remove *Bacillus* spores from spacecraft hardware materials. Astrobiology **4:**377–390.
68. **Venkateswaran, K., N. Hattori, M. T. La Duc, and R. Kern.** 2003. ATP as a biomarker of viable microorganisms in clean-room facilities. J. Microbiol. Methods **52:**367–377.
69. **Venkateswaran, K., M. Kempf, F. Chen, M. Satomi, W. Nicholson, and R. Kern.** 2003. *Bacillus nealsonii* sp. nov., isolated from a spacecraft-assembly facility, whose spores are gamma-radiation resistant. Int. J. Syst. Evol. Microbiol. **53:**165–172.
70. **Venkateswaran, K., M. Satomi, S. Chung, R. Kern, R. Koukol, C. Basic, and D. White.** 2001. Molecular microbial diversity of a spacecraft assembly facility. Syst. Appl. Microbiol. **24:**311–320.
71. **Vreeland, R. H., W. D. Rosenzweig, and D. W. Powers.** 2000. Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. Nature **407:**897–900.
72. **Wrenn, B. A., and A. D. Venosa.** 1996. Selective enumeration of aromatic and aliphatic hydrocarbon degrading bacteria by a most-probable-number procedure. Can. J. Microbiol. **42:**252–258.